IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Examiner: KELLY, Robert M.
QI *et al*	Art Unit: 1633
Serial No. 10/804,762	
Filing Date: March 19, 2004	
For: *Specific Inhibition of Allorejection*	

DECLARATION
Pursuant to § 1.132

The undersigned, Dr. Uwe Staerz, hereby declares as follows:

1. I received my Ph.D. in Immunology in 1986. A copy of my curriculum vitae is attached. I am employed by Isogenis, Inc., the assignee of the above-referenced application and currently serve Chief Scientific Officer of the company.

2. I have read and am familiar with the disclosure in the above-referenced application and have reviewed as well the Examiner's comments in his most recent office action mailed May 2, 2006. I understand that the Examiner is concerned about the level of CD8 alpha chain expression in the allograft tissues and how it correlates with the immune inhibitory effect. As described in more detail herein, our own data in a solid organ transplant model demonstrates that only a fraction of the allograft cells actually need express the CD8 alpha chain in order for the immune inhibitory effect to be achieved *in vivo*.

3. In the experiment described in the patent specification at Example 3, standard pancreatic islet purification protocols were used. Adenoviral veto vectors had been produced as Adenoviral (Type 5) vectors. They were replication-deficient due to a lack of the E1 region (DE1). Genes coded within the E3 region had also been deleted to avoid the down-regulation of the MHC class I molecules on infected cells. The Adenoviral veto vector mAdCD8 carried the mouse CD8 alphachain as transgene, whose expression was controlled by a CMV immediate early promotor/enhancer.

4. Transduction conditions were optimized for the Adenoviral veto vector mAdCD8. An 18-hr transduction at the relatively low overall MOI of 3/1 resulted in efficient transduction and high viabilities of the small solid pancreatic islets. A control vector of similar composition carried beta-galactosidase as transgene. Immunohistology studies revealed that under these conditions approximately 50% of pancreatic islets showed detectable levels of the CD8 alphachain (Figure 1). In single cell suspensions approximately 18% of islet cells scored positive for surface expression (FACS, EPICS Coulter). The immunofluorescence staining of viable pancreatic islets also demonstrated that expression of the CD8 alphachain was especially found on cells of the surface layer of the pancreatic islets. The outside layer of cells efficiently expressed the veto molecule thus providing a barrier layer of CD8 alphachain expressing cells.

5. As described in the subject application, in control experiments mock-infected C57Bl/6 pancreatic islets were transplanted under the kidney capsule of streptozotocin-treated Balb/c mice. As seen in Figure 2, these islets were rejected. Another group of Balb/c recipients received C57Bl/6 pancreatic islets that had been transduced with mAdCD8. These animals received no additional immune suppressive therapy. mAdCD8-infected pancreatic islets were no longer rejected (Figure 2). After 45 weeks in the recipient animal, the overall histology of transduced allogeneic pancreatic islets was similar to that of islets transplanted into syngeneic hosts (Figure 3). Expression of the CD8 alphachain could be detected in some pancreatic islets.

6. Accordingly, as our data demonstrates, expression of CD8 alpha chain by every transplanted allograft cell is not required in order for the subject therapy to work *in vivo*, even with a solid organ transplant. Efficient transfection of the outside layer of allograft cells in the solid organ is sufficient to interact with responding T cells and inhibit their activity.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Uwe D. Staerz

09-24-06
Date

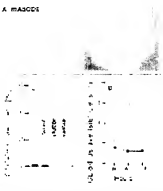

Figure 2: Purified pancreatic islets were transduced with mAdCD8 (A-rec in D) or AdLacZ (B, black, n=3) for 18-hrs, incubated for an additional 24-hrs and evaluated immunohistologically for the expression of CD8. A peroxidase-labelled anti-CD8 antibody was used (n/A) and a FITC-labelled one (B) (C) and (D).

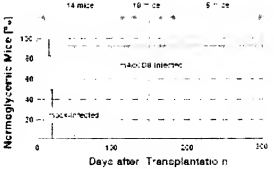

Figure 2 Survival of mock-infected and mAdCD8-transduced C57Bl/6 pancreatic islets in Balb/c mice.

Figure 3: C57Bl/6 mock-infected (A) or mAdCD8-transduced (B) pancreatic islets were transplanted into C57Bl/6 (A) or Balb/c (B) recipients. Anti-CD8 immunoperoxidase H&E staining.

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EDUCATION

University Training:

1977:	Eberhard-Karls-Universitaet, Tuebingen, Germany B.S.; Premed
1979:	Eberhard-Karls-Universitaet, Tuebingen, Germany B.S.; Biochemistry
1982:	Eberhard-Karls-Universitaet, Tuebingen, Germany M.D.
1986:	University of California, San Diego, CA, USA Ph.D.

AWARDS

1975-1982:	Studienstiftung des Deutschen Volkes
1983:	Deutscher Akademischer Austauschdienst

MEDICAL LICENSE

1982:	Federal Republic of Germany

EXPERIENCE

2000-	Chairman, Chief Scientific Officer, ISOGENIS, Inc., Denver Colorado
2004-	Senior Faculty Member, Institute for Therapeutic Biology, Denver, Colorado
2000 - 2003	Senior Faculty Member, National Jewish Medical and Research Center, Denver, Colorado
2000 - 2005	Professor, Department of Immunology, University of Colorado Health Sciences Center, Denver, Colorado

1995 - 2000 Associate Member, Department of Medicine, National Jewish Medical and Research Center, Denver, Colorado

1995 - 2000 Associate Professor, Department of Immunology, University of Colorado Health Sciences Center, Denver, Colorado

1990 - 2005 Member, Cancer Center, University of Colorado Health Sciences Center, Denver, Colorado

1994-1995 Interim Director of Animal Care Facility at the National Jewish Medical and Research Center, Denver, Colorado

1990-1994 Assistant Professor, Department of Microbiology and Immunology, University of Colorado Health Sciences Center, Denver, Colorado

1989-1994 Assistant Member, Department of Medicine, National Jewish Medical and Research Center, Denver, CO, USA

1986-1989 Member, Basel Institute for Immunology, Basel, Switzerland

1983-1986 Research Fellow, Scripps Clinic and Research Foundation, La Jolla, California

1982: Medical Resident in Clinical Pathology, Labor Dr. P. Schulz, Ludwigsburg, Germany

PROFESSIONAL MEMBERSHIPS

Member Review Committees NIH: Program Project on New Methods of Immune Intervention, on Thymic Involution, on Tuberculosis Diagnostics, on T Cell Development, on Vaccine Development and on Xenotransplantation

Member Review Committee Department of the Army: Prostate Cancer Treatment

Ad Hoc Member, Immunobiology Study Section, GDD Study Section

Ad Hoc Member, Immunology Review Committee - American Cancer Society

Associate Editor, Journal of Clinical Immunology

PATENTS

US Patent 5,078,998:

Hybrid Ligand Directed to Activation and Cytotoxic Effector T Lymphocytes and Target Associated Antigen

US Patent 6,060,054 - Product for T lymphocyte Immunosuppression;

US Patent 6,264,950 - Product and Process for T lymphocyte Immunosuppression

PUBLICATIONS

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